Published online April 25, 2006

A Comparison of U.S. and Chinese Sorghum Germplasm for Early Season Cold Tolerance

Cleve D. Franks,* Gloria B. Burow, and John J. Burke

ABSTRACT

Early season cold tolerance in grain sorghum [*Sorghum bicolor* (L.) Moench] is a desirable trait for extending its production range and minimizing risks associated with early spring plantings. Ten Chinese Kaoliang accessions were compared with 10 U.S. inbred parental lines and 10 U.S. commercial hybrids for a range of cold tolerance traits under laboratory, growth chamber, and field settings. Chinese lines were superior to both the inbred and hybrid classes in laboratory germination rates and field-based rates of emergence. In the growth chamber assays, Kaoliangs were not significantly different than U.S. hybrids for most traits measured at either of the two temperature treatments (12 and 24°C), with the exception of shoot length, for which the Chinese germplasm was higher. At the cooler temperature, Kaoliangs were significantly greater than U.S. inbreds for only fresh shoot weight; when tested at the warmer temperature, Kaoliangs had higher dry root weight, fresh and dry shoot weights, and fresh and dry whole plant weights, relative to the U.S. inbred class. The U.S. hybrids had greater total plot weight and final stand counts in the field than Kaoliangs, which were likewise higher than U.S. inbreds for both of these traits. Chinese accessions from this working group would serve as a source of favorable genes primarily for tolerance to low temperatures during the germination and emergence phase of growth in the breeding of cold tolerance sorghum lines.

In recent years, there has been increased interest in the development of grain sorghum germplasm adapted to U.S. production systems with heightened levels of tolerance to early season cold temperatures. This germplasm could serve both to expand the geographical range of grain sorghum cultivation and minimize the inherent risks involved in early season planting of grain sorghum within sorghum production regions. Additionally, an earlier sowing date could offer growers the option of capitalizing on higher levels of available soil moisture in the early spring and lower evapotranspirative demands, and thus, could potentially serve as a drought avoidance strategy.

The initial phase of any plant improvement program is the identification of superior germplasm for the trait of interest (Stoskopf, 1993). With respect to early season cold tolerance in sorghum, Chinese sorghum germplasm of the working group Nervosum-Kaoliang has historically been reported as being superior in this regard. There is ample anecdotal evidence of this class of germplasm possessing heightened levels of early season cold tolerance, but specific measures of the germination and seedling vigor qualities of this germplasm as a class are somewhat limited. Stickler et al. (1962), in comparing a small set of Chinese and U.S. germplasm, found that the Kaoliangs were typically superior in terms of germination and emergence under suboptimal temperatures and indicated that these lines might also possess some advantage in terms of their seedling growth rate across all temperatures tested. Qingshan and Dahlberg (2001) examined a large number of Chinese accessions and noted that several accessions within the Chinese germplasm collection showed high levels of both germination under cold temperatures and seedling phase cold tolerance.

It has been postulated, in both sorghum and maize (*Zea mays* L.), that the germination–emergence and early growth stage phases of seedling development are each under the control of entirely different suites of genes (Cisse and Ejeta, 2003; Hodges et al., 1997; Nordquist, 1971). In breeding for overall early season cold tolerance, therefore, it may be necessary to evaluate germplasm sources for tolerance in each phase. This study was undertaken to examine a set of Chinese sorghum lines in laboratory, growth chamber, and field settings for both cold temperature germination and seedling vigor traits and to compare the response of these lines with a representative set of U.S. parental lines and commercial hybrids.

MATERIALS AND METHODS

Germplasm Utilized

The sorghum lines and hybrids included in this study were 10 typical accessions of three different classes of germplasm: (i) Chinese landrace accessions of the working group Nervosum-Kaoliang; (ii) Publicly available grain sorghum inbred lines from the Texas Agricultural Experiment Station sorghum breeding program; and (iii) U.S. grain sorghum hybrids provided by seed companies (Table 1). All seeds were treated with captan (*N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide; Drexel Chemical Company, Memphis, TN), Concep III (fluxofenim, 4′-chloro-2,2,2-trifluoroacetophenone *O*-1,3-dioxolan-2-ylmethyloxime; Novartis Crop Protection, Inc., Greensboro, NC), and Apron XL [metalaxyl-m. methyl *N*-(methoxyacetyl)-*N*-(2,6-xylyl)-D-alaninate; Syngenta Crop Protection, Greensboro, NC] in liquid form at label rates before testing.

Laboratory Tests

To evaluate germination response among the entries, a "G50" index was utilized as described by Mann et al. (1985). For each entry, 50 seeds were planted in filter paper-lined 50-mm Petri dishes at each of eight temperatures ranging from 10 to 24°C (±0.5°C) in two degree increments. Each 50-seed treatment was placed on one of eight individual thermal plates designed to maintain a separate constant temperature (Burke and Mahan, 1993), and 3 mL of distilled H_2O were added to

USDA-ARS Plant Stress & Germplasm Development Unit, Cropping Systems Research Laboratory, Lubbock, TX 79415. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. Received 25 Aug. 2005. *Corresponding author (cfranks@lbk.ars.usda.gov).

Published in Crop Sci. 46:1371–1376 (2006).
Plant Genetic Resources
doi:10.2135/cropsci2005.08-0279

677 S. Segoe Rd., Madison, WI 53711 USA

Table 1. Lines and hybrids included in study, their origins, G50 values, and respective standard errors.

Line	Type	Origin	G50	S. E.
PI 563849	Kaoliang	Beijing, China	13.39	0.12
PI 567939	Kaoliang	Beijing, China	13.99	0.18
PI 567943	Kaoliang	Beijing, China	13.22	0.06
PI 567944	Kaoliang	Beijing, China	15.21	0.22
PI 567946	Kaoliang	Beijing, China	13.89	0.22
PI 563559	Kaoliang	Liaoning, China	13.50	0.16
PI 610727	Kaoliang	Shanxi, China	12.39	0.15
PI 567962	Kaoliang	Shanxi, China	14.68	0.20
PI 568015	Kaoliang	Shanxi, China	14.13	0.21
PI 568024	Kaoliang	Shanxi, China	14.05	0.24
BTx399	U.S. parental	Texas Agricultural Experiment Station	16.09	0.28
BTx623	U.S. parental	Texas Agricultural Experiment Station	16.00	0.14
BTx642	U.S. parental	Texas Agricultural Experiment Station	15.34	0.19
BTx643	U.S. parental	Texas Agricultural Experiment Station	16.03	0.16
BTx3042	U.S. parental	Texas Agricultural Experiment Station	16.47	0.23
RTx430	U.S. parental	Texas Agricultural Experiment Station	16.71	0.15
RTx436	U.S. parental	Texas Agricultural Experiment Station	16.67	0.12
Tx2737	U.S. parental	Texas Agricultural Experiment Station	17.02	0.37
Tx2783	U.S. parental	Texas Agricultural Experiment Station	17.67	0.21
Tx2817	U.S. parental	Texas Agricultural Experiment Station	15.14	0.09
GT-97619	U.S. hybrid	Garrison & Townsend Seed Company	15.32	0.12
GT-SG822	U.S. hybrid	Garrison & Townsend Seed Company	17.59	0.37
GT-SG942	U.S. hybrid	Garrison & Townsend Seed Company	15.11	0.09
Pioneer Hybrid 8500	U.S. hybrid	Pioneer Hybrid Seed Company	15.50	0.17
Pioneer Hybrid 85G85	U.S. hybrid	Pioneer Hybrid Seed Company	16.80	0.21
Pioneer Hybrid 85Y34	U.S. hybrid	Pioneer Hybrid Seed Company	15.46	0.10
Pioneer Hybrid 86G71	U.S. hybrid	Pioneer Hybrid Seed Company	17.60	0.10
TR420	U.S. hybrid	Triumph Seed Company	16.98	0.20
TRX20261	U.S. hybrid	Triumph Seed Company	15.11	0.16
TRX94893	U.S. hybrid	Triumph Seed Company	16.56	0.22
All Kaoliangs			13.83a	0.01
All parental lines			16.33b	0.01
All hybrids			16.19b	0.01

each dish. The seeds were allowed to germinate in the dark for 48 h, at which time the total number of seeds germinated per temperature treatment was determined. Germination was defined as the extension of the radicle or coleoptile at least 1 mm beyond the seed coat. Each eight temperature germination experiment was considered as a single replication, and two replications per line were performed. The number of seeds germinated at a given temperature was divided by the maximum number of seeds germinated at any one temperature for that experiment to give the value y. Calculation of G50 values was as described under the Statistical Analysis section below.

To test seedling vigor at cool temperatures, 10 seeds of each entry were sown in “rag dolls,” consisting of two layers of germination paper (Anchor Paper, St. Paul, MN), which were rolled into a single layer of wax paper and placed in a glass jar. Ten seeds per replication were aligned 1 cm from the upper edge of the germination paper. Distilled water was added to the jars and allowed to wick up to the level of the seeds. To reduce the effects of differences in cold germination among the lines, the jars were placed in a growth chamber at 24°C for 48h with a 12-h day/night cycle, after which time the temperature was reduced to 12°C. After 8 d, seeds and seedlings were removed from the rag dolls, aligned on a laminated sheet with centimeter gradations, and photographed for the purposes of measuring seedling lengths. Roots and shoots were separated at this time, and fresh weights of each individual treatment were taken. Plant materials were dried at 80°C for at least 7 d, and dry weights for all entries were determined. All lines were also tested in an identical fashion at a constant 24°C with a 12-h day/night cycle for 8 d for purposes of comparison. Variables recorded for each replication included shoot lengths of all germinated seeds, total shoot weights (fresh and dry), and total root weights (fresh and dry). Fresh and dry whole plant weights were calculated as the sum of the root and shoot weights. Three replications per line at both temperatures were performed.

Field Trial

All 30 entries were planted in a completely randomized block with three replications in Lubbock, Texas, on April 1, 2004. Plots lengths were 4.67 m, and row spacing was 1.02 m. Each replication consisted of 100 seeds planted 3 cm deep with a John Deere MaxEmerge Planter modified for use in small plot research. Soil type was an Amarillo fine sandy loam. Emergence data was taken every second day, and an Emergence Index for each plot was calculated as $\Sigma\ (E_j \times D_j)/E$, where E_j = emergence on day j, D_j = days after planting, and E = final stand (Smith and Millett, 1964). Total emergence counts and total plot dry weight was recorded on 2 May 2004. Air temperatures at 5-min intervals were recorded for the duration of the experiment via a weather station adjacent to the test plots.

Statistical Analysis

The calculation of the G50 value was performed as per Mann et al. (1985), in which nonlinear regression was used to derive the G50 value, an estimate of the minimum temperature at which 50% of a seed lot can be expected to germinate within a given time period, in this case 48 h. The nonlinear regression formula $E\ (y) = \beta_0\ [1 + e^{-(\beta_1+\beta_2 x)}]^{-1}$ was used to calculate the G50 value for each experiment, where G50$=-\beta_1/\beta_2$. Variance of the G50 values for a given line or category were calculated as $[\beta_1^2 \text{ var } (\beta_2)/\beta_2^2 - 2\beta_1 \text{ cov } (\beta_1,\beta_2)/\beta_2 + \text{var } (\beta_1)]/\beta_2^2$, from which the standard error values were derived. This value was shown to be strongly correlated with the base germination temperature of a given line, and offers the advantage of higher throughput over the traditional method of determining base temperature via longer term germination counts (Mann et al., 1985).

At each of the eight germination temperature regimes, the three germplasm classes were compared in terms of their germination percentage. Since data in the form of percentages

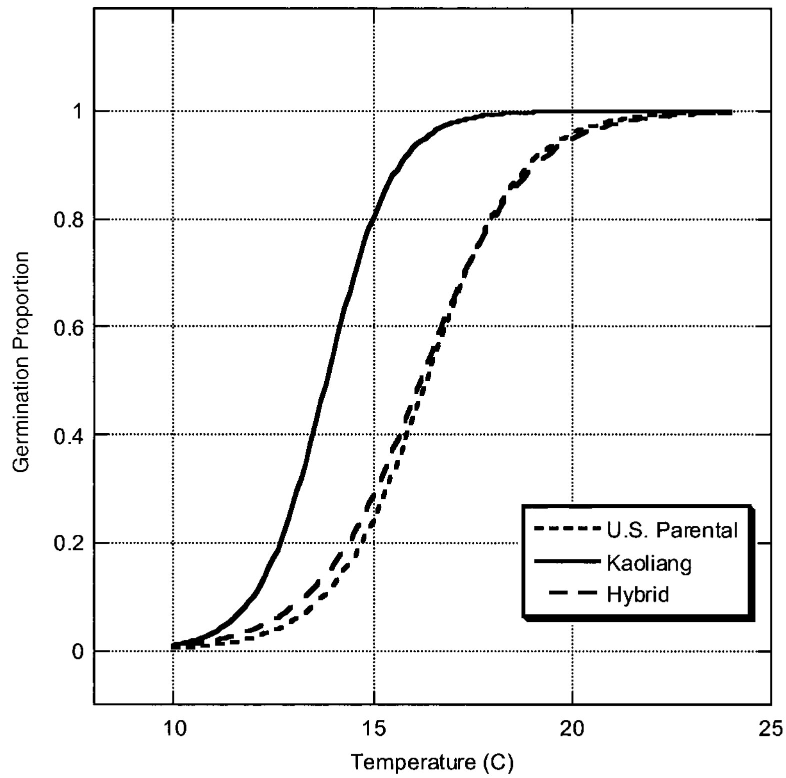

Fig. 1. Mean proportion of seed germinated within 48h across a range of temperatures, by germplasm class.

frequently suffers from heterogeneity of error variances, an arcsine transformation was used on all germination percentages. The model for this analysis was $y' = C_i + R_j + E_{ij}$, where y' = arcsine-transformed value of germination percentage, C_j = germplasm class, R_j = replication, and E_{ij} = random error term associated with both germplasm class and replication. Each replication in this analysis consisted of two replications of each of the 10 entries per germplasm class, for a total of 20 replications per class for each of the eight temperature treatments. Mean separation of treatment means was conducted by Tukey's HSD.

RESULTS

On the basis of G50 values, the Chinese germplasm was quite superior to U.S. grain sorghum lines and hybrids, with nine of the 10 best entries being Kaoliangs (Table 1). For all temperatures tested below 20°C, the Kaoliangs were significantly superior in terms of their germination percentage while the U.S. inbred lines and hybrids were not significantly different from each other at any temperature included in this study (Fig. 1; Table 2).

In the growth chamber tests of early season vigor, there were no significant differences between the Kaoliang and the U.S. hybrid germplasm classes with the exception of mean shoot length (Table 3). At both temperatures, the Kaoliangs had significantly longer shoots than all U.S. germplasm. The Kaoliangs were superior to U.S. inbreds for dry root weight at 24°C, fresh shoot weight at both 12 and 24°C, dry shoot weight at 24°C, and both fresh and dry whole plant weights at 24°C.

Differences among the germplasm classes were much more pronounced in the field trial. For both whole plot weight and final stand counts, hybrids were superior to Kaoliangs, which in turn showed significantly higher values than the U.S. parental lines. With respect to rate of emergence, as measured by the emergence index, the Kaoliangs showed faster emergence rates than the U.S. hybrids, and the hybrids had significantly faster emergence rates than the parental lines.

There were significant correlations between growth chamber and field data for most variables, with the exceptions of dry root weight at 12°C, mean shoot length at 12°C, and the G50 value (Table 4). Mean shoot length at 12°C and the G50 value, however, were both significantly correlated with the field emergence index. For

Table 2. Mean germination percentages, by germplasm class, and temperature. Nontransformed percentages are reported, mean separation conducted by arcsine transformed variables. Each mean was based on 20 replications of 50 seeds at each temperature. Means within rows not connected by same letter are significantly different (α = 0.05).

	Mean germination percentage		
Temperature	**U.S. hybrids**	**U.S. parental lines**	**Kaoliangs**
°C			
10	**0.0b**	**0.0b**	**0.5a**
12	**0.7b**	**0.2b**	**7.1a**
14	**15.5b**	**7.7b**	**57.5a**
16	**47.4b**	**46.4b**	**90.0a**
18	**80.6b**	**79.4b**	**93.7a**
20	**91.6a**	**90.2a**	**96.2a**
22	**95.4a**	**95.7a**	**97.1a**
24	**98.7a**	**96.6a**	**98.2a**
Combined	**71.8b**	**68.9b**	**92.9a**

Table 3. Mean values of measured variables by germplasm category. Means not connected by same letter are significantly different (α = 0.05).

		12°C			24°C		
	Variable	U.S. hybrids	Kaoliangs	U.S. inbreds	U.S. hybrids	Kaoliangs	U.S. inbreds
Growth chamber	fresh root weight	0.411a	0.356a	0.328a	0.816a	0.762ab	0.601b
	dry root weight	0.049a	0.043a	0.044a	0.077a	0.082a	0.041b
	fresh shoot weight	0.454a	0.440a	0.349b	0.701ab	0.779a	0.598b
	dry shoot weight	0.080a	0.076a	0.051a	0.134a	0.127a	0.077b
	fresh whole plant weight	0.871a	0.796a	0.697a	1.52a	1.54a	1.20b
	dry whole plant weight	0.130a	0.119a	0.096a	0.211a	0.210a	0.117b
	mean shoot length	5.00b	5.89a	4.67b	7.05b	8.38a	6.44b
Field	whole plot weight	8.00a	4.76b	2.15c			
	final stand	59.33a	45.03b	30.63c			
	emergence index	17.08b	16.20c	18.01a			

field whole plot weight, the growth chamber variable with the highest coefficient of correlation was fresh whole plant weight at 12°C (r = 0.70).

DISCUSSION

The G50 estimate is an attempt to describe the germination of a given genotype across a range of temperatures with a single statistic, and as such, is inherently limited. Examining germination fractions across a range of temperatures (Fig. 1) reveals that the Kaoliangs began germinating at lower temperatures than the U.S. germplasm, and reached a maximal plateau of germination at a substantially lower temperature threshold. This finding is supported by the individual analyses of germination proportions across temperatures (Table 2). It is also apparent from both the G50 values and the lack of significant differences between U.S. hybrids and inbred lines at the temperatures examined that there was little inherent heterotic advantage with respect to germination for the hybrids included in this study.

The higher germination rates and lower germination temperature thresholds of the Kaoliangs apparently did not, however, translate into a measurable advantage in early season field performance in terms of biomass production. The only field variable to which the G50 value was significantly correlated was the emergence index. These results tend to support the previously stated supposition that germination and rate of emergence are genetically distinct from early season vigor.

The longer shoot lengths may in part be due to the fact that most Kaoliangs, including all of those represented in this study, are one or two dwarf in stature, whereas all of the U.S. germplasm in this study was of typical grain sorghum combine height (three dwarf). Further experimentation is underway to determine the effects of dwarfing genes on early season vigor.

For growth chamber data, the general numeric trend for both fresh and dry weights of plant components was that hybrids were superior, followed by the Kaoliangs, with U.S. inbreds typically last. It is important to recognize the potential contribution of heterosis in this comparison. Yu and Tuinstra (2001) noted favorable heterotic effects in sorghum for early season vigor traits in a study involving a range of cold-tolerant and susceptible germplasm in a Design II mating scheme. Tiryaki and Andrews (2001) also observed both positive and negative specific combining ability in a set of diverse lines crossed to common testers. It remains to be determined whether the cold tolerance superiority of the Kaoliangs in this study would be expressed in hybrid combinations, but their relative merit when compared with U.S. hybrids without the benefit of heterosis is a promising observation.

At 12°C, the Kaoliangs were significantly superior to U.S. inbreds in terms of growth rate only for total fresh

Table 4. Genotype mean correlations among growth chamber, laboratory, and field variables.

	Measurement	Plot weight	Final stand count	Emergence index
		g		
Growth chamber data	fresh root weight, 12°C	0.66**	0.52**	−0.47**
	fresh root weight, 24°C	0.63**	0.59**	−0.60**
	dry root weight, 12°C	0.11	−0.01	0.04
	dry root weight, 24°C	0.43*	0.42*	−0.47**
	fresh shoot weight, 12°C	0.67**	0.61**	−0.63**
	fresh shoot weight, 24°C	0.53**	0.48**	−0.67**
	dry shoot weight, 12°C	0.66**	0.57**	−0.51**
	dry shoot weight, 24°C	0.50**	0.55**	−0.59**
	fresh whole plant weight, 12°C	0.70**	0.59**	−0.58**
	fresh whole plant weight, 24°C	0.61**	0.57**	−0.66**
	dry whole plant weight, 12°C	0.66**	0.52**	−0.44*
	dry whole plant weight, 24°C	0.63**	0.65**	−0.71**
	shoot length, 12°C	0.08	0.01	−0.50**
	shoot length, 24°C	0.41*	0.42*	−0.75**
	G50	−0.05	−0.12	0.64**
Field data	Plot Weight		0.89**	−0.51**
	Final Stand Count			−0.61**

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.

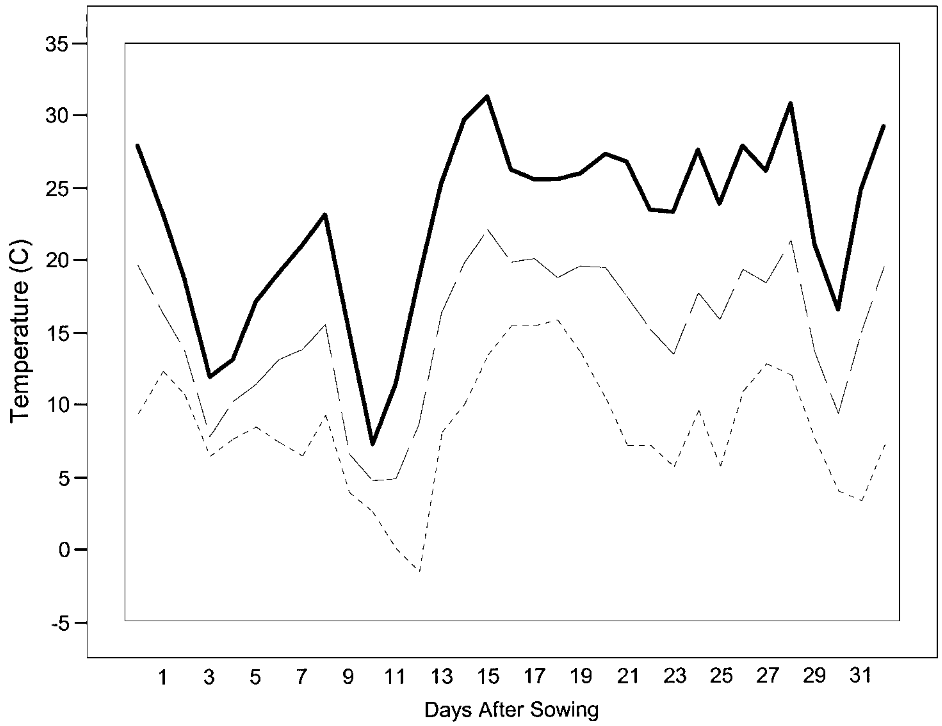

Fig. 2. Daily maximum, minimum, and average air temperatures, by day after sowing during field observation period. Termination of lines indicates point at which plots were harvested.

shoot weight and shoot length, but displayed significantly higher values for five of the measured variables in the 24°C treatment. This would seem to indicate a greater inherent growth rate within the Chinese germplasm group, which perhaps was heightened by the increase in temperature between the two treatments. If such is the case, then testing for early season vigor under warmer conditions might be nearly as effective as doing so under conditions of cold stress.

The minimum air temperatures observed during the field testing phase of this study were sufficiently low to qualify the nursery as a successful early season cold tolerance nursery (Fig. 2). Indeed, the daily minimum air temperatures seldom exceeded 10°C, and even dropped to below freezing before the emergence of the seedlings. The relatively high emergence index values are indicative of the suboptimal soil temperatures to which the seeds were exposed. It can also be noted, however, that the observed daily high temperatures were well above 20°C for much of this period, which in all likelihood contributed to the relatively strong correlations between the 24°C growth chamber and field measurements in most cases.

The significant correlations between a large number of the variables observed in the growth chamber and those collected in the field indicate that the growth chamber is a suitable proxy for early season vigor field trials. Although no single variable explained all of the variation encountered in the field trial, the strong correlations observed offer promise for the use of the growth chamber as a preliminary tool for the development of cold tolerant sorghum germplasm. Further indications of the utility of controlled environment conditions in predicting field responses have been shown by Brar and Stewart (1994) and Yu et al. (2001).

While it appears that sorghum accessions of the working group Nervosum-Kaoliang do indeed hold promise as a source of cold tolerance, especially for germination and rate of emergence under cool conditions, it should be pointed out that most lines within this class are extremely exotic and unadapted to U.S. grain sorghum production schemes. This will undoubtedly present a considerable challenge to sorghum breeders desiring to incorporate these genes into adapted germplasm.

ACKNOWLEDGMENTS

The authors would like to thank Charles Woodfin and Julia Veyro for their excellent technical support and Dr. Edward Gbur for his helpful input on statistical analysis.

REFERENCES

Brar, G.S., and B.A. Stewart. 1994. Germination under controlled temperature and field emergence of 13 sorghum cultivars. Crop Sci. 34:1336–1340.

Burke, J.J., and T.J. Mahan. 1993. A controlled, eight-position, thermal plate system for physiological investigations. Appl. Eng. Agric. 9(5):483–486.

Cisse, N., and G. Ejeta. 2003. Genetic variation and relationships among seedling vigor traits in sorghum. Crop Sci. 43:824–828.

Hodges, D.M., C.J. Andrews, D.A. Johnson, and R.I. Hamilton. 1997. Sensitivity of maize hybrids to chilling and their combining abilities at two developmental stages. Crop Sci. 37:850–856.

Mann, J.A., E.E. Gbur, and F.R. Miller. 1985. A screening index for adaptation in sorghum cultivars. Crop Sci. 25:593–598.

Nordquist, P.T. 1971. Use of earliness and cold tolerance in breeding sorghums. Proc Annu. Corn and Sorghum Research Conference. 26:33–41.

Qingshan, L., and J.A. Dahlberg. 2001. Chinese sorghum genetic resources. Econ. Bot. 55(3):401–425.

Smith, P.G., and A.J. Millett. 1964. Germinating and sprouting response fo the tomato at low temperatures. J. Am. Soc. Hortic. Sci. 84:480–484.

Stickler, F.C., A.W. Pauli, and A.J. Casady. 1962. Comparative responses of Kaoliang and other grain sorghum types to temperature. Crop Sci. 2:136–139.

Stoskopf, N.C. 1993. Plant breeding: Theory and practice. Westview Press, Inc., Boulder, CO.

Tiryaki, I., and D.J. Andrews. 2001. Germination and seedling cold tolerance in sorghum: II. Parental lines and hybrids. Agron. J. 93: 1391–1397.

Yu, J., and M.R. Tuinstra. 2001. Genetic analysis of seedling growth under cold temperature stress in grain sorghum. Crop Sci. 41: 1438–1443.

Yu, J., M.R. Tuinstra, M.M. Claassen, W.B. Gordon, and M.D. Witt. 2001. Analysis of cold tolerance in sorghum under controlled environment conditions. Field Crops Res. 85:21–30.